Bacterial contamination of toothbrushes with comparison of healthy and dental patients

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Abstract

Twenty four normal toothbrushes were tested for adult persons ,12 brushes used by healthy individual and 12 brushes used by patient of oral infection(gingivitis or periodontitis) each brush was used for at least 5 weeks period .

Both brushes of two groups were colonized by large number of organisms ranged from 0.2×10^2 to 3.5×10^2 C.F.U/ml on healthy individual brushs and from 2.8×10^2 to 5×10^2 C.F.U/ml on patient brushes.

Each brushes of healthy individual yielded various types of organisms as *Pseudomonas, Staphylococcus epidermidis, Staph.aureus*, Gram positive rods and yeast but most brushes of patients yielded one type of organisms.

T- test analysis appeared that there were high significant difference at ($P < 0.05$) between brushes of two groups in the total mean of different organisms that isolated from them.

Pseudomonas recorded highest proportion (57% of total organisms isolated on all brushs of two groups; 83% of brushs) followed by *Staphylococcus* (36% of total isolated organisms ; 58% of brushs) Gr+ve recorded lowest proportion(3% of total isolated organisms ; 33% of brushs) .
Staph. epidermidis , *Staph. aureus* could isolate from brushes of patient in 6 days after brushing while *Pseudomonas* isolated after 3 days.

This study demonstrated *Staphylococcus* and *Pseudomonas* as pathogen agent that cause oral infections and conclude that toothbrushes may be as a source of opportunistic pathogen such these microorganisms by wrong storing ways or by the same infected person .

Introduction

The human oral cavity is colonized by a larger variety of bacteria flora than any other anatomic area .more than 700 species of bacteria have already been identified 400 of which were found in the periodontal pocket adjacent to teeth (Abraham *et al*.,1990) . Organisms not normally associated with oral flora also have been isolated from toothbrushes ,including enterobacteria, *Pseudomonas* (Sammons *et al*.,2004 So the infectious microorganisms remaining on the brush can reinfect our mouth teeth again ,some of them can even spread to the rest of our body and cause serious health problems ,including heart disease, stroke ,arthritis ,haematogenous , bacterimia and chronic (Warren *et al*., 2001; Sammons *et al*.,2004). There are many ways allows the bacteria bread and grow on toothbrushes ,spray from flushing toilet, adamp environment, a single toothbrush can be the breeding ground for tillions of bacteria(Abraham *et al*.,1990).

There are attempt to reduce bacterial survival time, deter colonization and inhibit biofilm formation by toothbrushes containing antibacterial agent have been developed and methods for sterilization of brushes devised (Caudry *et al*.,1995 ; Neal & Rippin, 2003). Particular attention was paid to *Staphylococci* and *Pseudomonas* like organisms as both of these are opportunistic pathogens responsible for many nosocomial infections and because *Pseudomonas* are also resistant to many disinfectants in toothpaste including triclosan(Warren *etal*.,2001). The aim of this study was to investigate and compare bacterial population on toothbrushes.

Materials and methods

Collection of samples

In this study (24) toothbrushes for adult individuals brushed with them for at least 5 weeks have been tested, (12) of them were for healthy individual (H.I)and (12) samples from adult person suffering from gingivitis or periodontitis as their doctors diagnosis.

Isolation of organisms

Toothbrush of every person were rinsed in tap water and transported to the laboratory in sterile bag, according to Sammons *et al*.(2004) handle of brush was cut off using a heat sterile scissors , head of the brush was then soaking in 10 ml of sterile tryptone soya broth (TSB), for 60 min ,followed by vortex mixing for 1 min and make swabbing to dislodge suspected adherent bacteria.

The bacterial suspension was one fold diluted for 10^{-1} and (0.1 ml) of broth plated by pipette into tryptone soya agar (TSA) , as non-selective media and into MacConkey , Manitol salt agar and Sabouraud's dextrose agar to isolate enterobacteria , Staphylococci and yeasts ,respectively,plates were incubated aerobically at 37C for 24- 48 h.

Identification:-

A total viable counts of bacterial population were enumerated ,colony colour , morphology and Gram's stain was performed for each isolates.

A.Gram positive cocci of Manitol salt agar were further identified as *Staphylococcus aureus* and *Staphylococcus epidermidis* by several biochemical tests:-

1-Catalase test(Collee *et al*.,1996) .

2-Oxidase test (Benson, 2002) .

3-Coagulase test(Collee *et al*.,1996) .

4. Acetoin production test (Stukus,1996).

5. Deoxy ribonuclase (DNAase) test (Collee *et al*.,1996) . 6.Carbohydrates fermentation test (Benson, 2002) .

B.Grame negative bacilli on MacConkey plates were identified as following:

a. Gram negative , non lactose fermenting , oxidase positive colonies were considered as *Pseudomonas spp*

b. Gram negative , lactose fermenting , oxidase negative colonies were considered as Coliform spp.

Survival of isolates on toothbrushes :-

After culture and characterization bacteria on brushes were diagnosed into of two groups , patient's brushes that labeled with (13,15,22) were selected for bacterial survival of *Staph. epidermidis, Staph. aureus*, *Pseudomonas* respectively.each person used these brushes provided with three new sterile brushes for brushing for at least 3 weeks then storage them in sterile bag for various period(24 h, 3 days, 6 days)(Sammons *et al*., 2004) .

Statistical analysis: Student t- test analysis was applied to determine the significance of differences at ($P<0.05$) between brushes types in total numbers for each types of organisms and in the total means.

Result and Discussion

Table 1: The total number of organisms on each brush of two groups.

H.I brushes	Total number of organisms C.F.U/ml	P. brushes	Total number of organisms C.F.U/ml
1 H	1.3×10^2	13P	3.5×10^2
2H	0.7×10^2	14P	1.5×10^2
3H	0.3×10^2	15P	4×10^2
4H	0.5×10^2	16P	3×10^2
5H	0.2×10^2	17P	3.3×10^2
6H	0.4×10^2	18P	2.7×10^2
7H	1.7×10^2	19P	2.8×10^2
8H	3.5×10^2	20P	5×10^2
9H	0.2×10^2	21P	3.2×10^2
10H	1.3×10^2	22P	4.5×10^2
11H	0.5×10^2	23P	4.2×10^2
12H	1.1×10^2	24P	2.8×10^2

P= Patient ' s sample H.I= Healthy individual' s sample

Table 2:The average total numbers for each microorganism and the total mean.

Microorganism	Average total numbers (C.F.U/ml) Healthy individual' s brushes	Patient's brushes	p.V (p<0.05)
Pseudomonas	5.9×10	18.75×10	0.0298 *
Staphyllococcus	2×10	13.66×10	0.022 *
G+ve rod	1.25×10	0.25×10	0.070 N.S
Yeast	0.5×10	1×10	0.062 N.S
Total mean	9.75×10	33.57×10	2.7×10^{-6} **

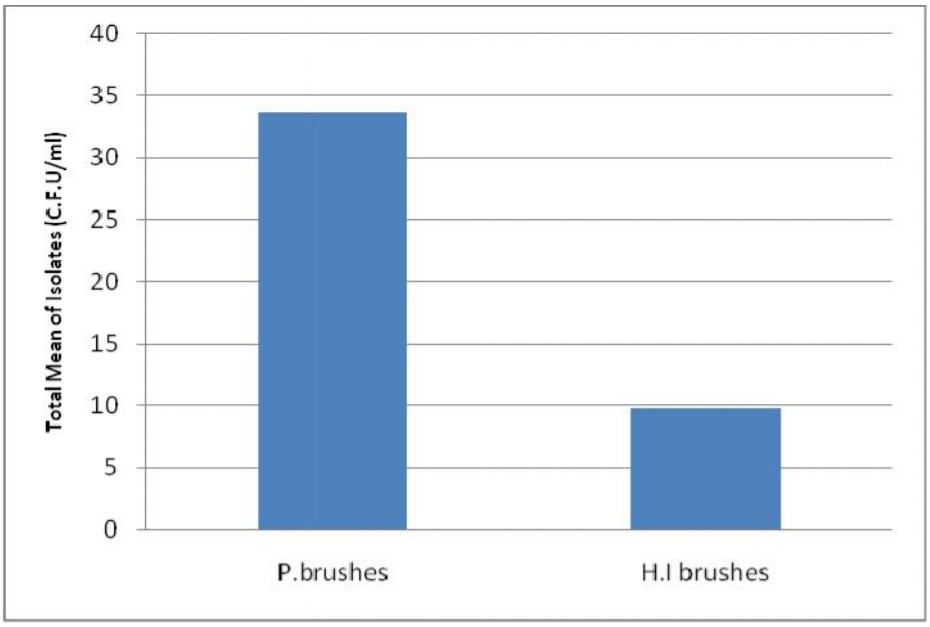
* = There were significant difference , N.S = No significant difference, ** = There were high significant difference

The total number of brushes that tested were 24, All brushes of two groups was yielded microbial colonies. There were variable in numbers of organisms on each brush but both brushes of healthy individual and patient were colonized by large number of organisms ranged from 0.2×10^2 to 3.5×10^2 C.F.U/ml on H.I brushs and from 1.5×10^2 to 5×10^2 C.F.U/ml on P. brushes,table.1The number of bacteria on brushes of patient was highest than those of H.I ones, these difference in bacterial load belong to presence oral inflammations(Taji *et al*., 1998)table1.

The total numbers of different types of isolates on brushes of each group was calculated, t - test showed there were significant difference at $p<0.05$ between brushes of two groups in the average total numbers of *Pseudomonas* and *Staphylococcus* , while no significant difference appeared for Gr+ve rod and yeast. From the other hand t-test also appeared high significant difference between the total mean of all types of organisms that isolated from brushes of each group, table 2, Fig 1.

All brushes of H.I yielded a mixed population of organisms , with one to four different types of colony on each brush,while most brushes of patients yielded one type of bacteria *Pseudomonas* or *Staphylococcus*, but with large number comparison with that on H.I brushes This may be due to the competition between different organisms and bacterial pathogens have evolved specific virulence factors that allow them to impair or kill other microbes (Nester *etal*.,2001).

Yeast were approximately isolated from half brushes of H.I in low numbers as flora, while from patient's brushes yeast isolated only from one case which was relatively in large number,it is seem the pathogen that cause infection. *Pseudomonas* was recorded the highest proportion of total organisms isolated on brushes of H.I and patient (61%,56%) respectively followed by *Staphylococcus* (21%,40%), always store toothbrush in closed container not in ventilated environment and keeping it in toilet place, causing of presence of these bacterial types on brushes because of these moisture environments is more stabilized when the brush is not aired (Caudry *et al*.,1995). comparison between relative proportion of different microorganisms that isolated from brushes of each group are shown in fig . 2.



Significant difference at $p < 0.05$

Fig 1: the total mean of all isolates on brushes of two groups

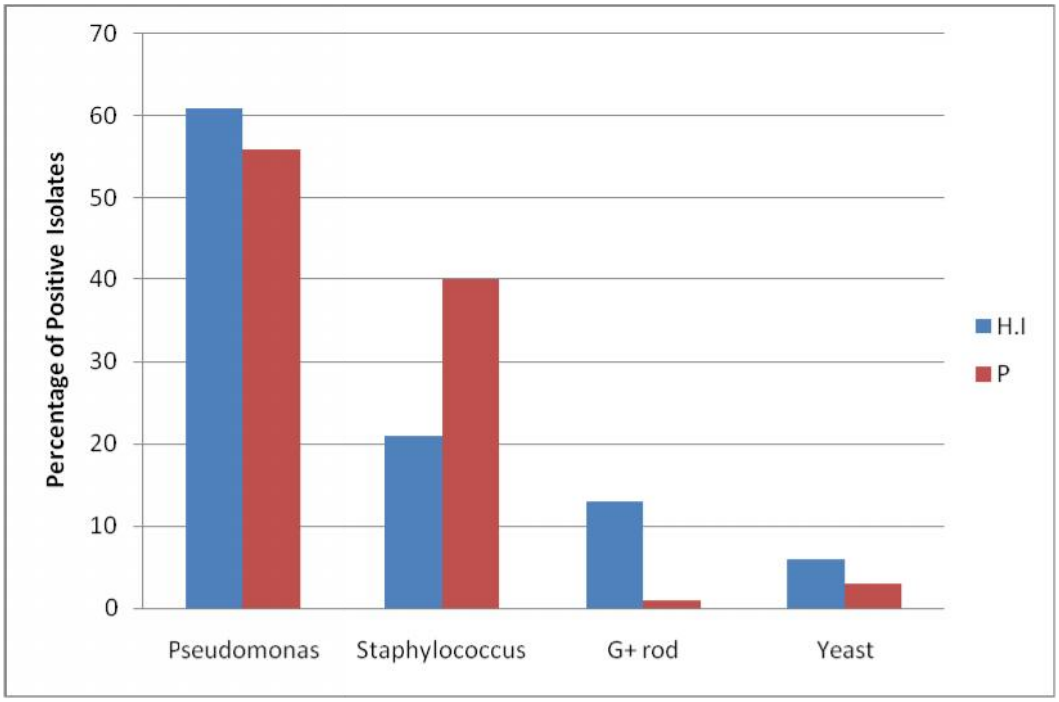


Fig:2 Percentage of microorganisms isolated from brushes of H.I and patients.

Table 3: Proportion of microorganisms isolated from all brushes and Percentage of positive brushes:

Isolates	**Percentage of positive brushes**	**Proportion of isolates**	**Range (C.F.U/ ml)**
Pseudomonas	**83%**	**57%**	**$1\times10 - 4.5\times10^2$**
Staphyllococcus	**58%**	**36%**	**$1\times10 - 4\times10^2$**
Staph.epidermidis	**79%**	**24%**	**$1\times10 - 3.5\times10^2$**
Staph. aureus	**21%**	**12%**	**$1\times10 - 4\times10^2$**
Gr+ve rod	**33%**	**3%**	**1×10 - 5×10**
Yeast	**29%**	**4%**	**1×10 - 2×10**

Out of the total (24) , 14 brush yielded bacterial growth on Manitol salt agar (58% of brushes) ; 3 brushes (21%) showed a growth of *Staphylococcus aureus*; 11 brush (79%) showed a growth of *Staphylococcus epidermidis* ,table 3.

Several previous studies have reported the isolation of *Staphylococcus* from toothbrushes(Alshayeb &Al- Ebrahim,2008; Gabe *et al*.,2011 ; Malmberg *et al*.,1994; Taji &Rogers,1998; Verran & Gilmartin, 1996). People can get Staphylococcal infection from contaminated objects , and can be spread from one area of the body to another if some one touches the infected area , share things like brush , towels, clothing. warm,humid environments can contribute to Staphylococcal infection.

Both *Staph.epidermidis* isolates were cultured from both brushes of two groups , but *Staph.aureus* isolates were cultured from only brushes of patient and just in three samples , it found in two samples of them with presence of large number of *Pseudomonas* . The result showed that *Staph. epidermidis* was that cause oral infection in three cases of patient individual these ensure the potential pathogencity of it. *Staph.aureu*s have also been recorded among isolates from toothbrushes in (Gabe *et al*.,2011; Smith *et al*.,2003; Taji &Rogers,1998).

Proportion of *Staph*. *epidermidis*(24%) of total organisms was larger in two time than these recorded by *Staph.aureus*(12%), table 3. These results differences compatible with (Alshayeb & Al-Ebrahim,2008) which recorded (26.6%),(20%) for *S.epidermidis ,S.aureus* respectively.

Pseudomonas ,Staphylococcus were isolated from 83% ,58% of all brushes respectively,it was more than 16%, 48% reported by (Sammons *et al*.,2004).

Pseudomonas are known to be resistant to triclosan (antibacterial agent is added to toothbrushes) (Van Delden& Iglewski,1998) may be for these reason *Pseudomonas* recorded highest proportion of (57%) of total organisms isolated on all brushs of two groups. Coliforms were not isolated in this study, although they isolated in other studies in different countries(Alshayeb & Al-Ebrahim,2008; Sammons *et al*.,2004).

yeasts were identified in 29% of brushes,Sammons *etal*.,2004 were identified no yeast and *Streptococcus* were rarely because of the aerobic culture condition ,in this study could not isolate *Streptococcus* for the same reason.

Table 4: Survival of *S.aureus ,S. epidermidis* and *Pseudomonas* isolated from selected brushes

Microorganism	**No. Sample**	**Total No.of bacteria**	**24hr**	**3 day**	**6 days**
S. epidermidis	**14**	**4×10**	**+**	**+**	**+**
S. aureus	**12**	**3.5×10^2**	**+**	**+**	**+**
Pseudomonas	**22**	**4.5×10^2**	**+**	**+**	**–**

Numbers of *S.aureus ,S. epidermidis* reduced by a factor of a proximately 10 after 24hr of storage and results of culture showed that growth of the two species were still viable after 6 days of storage,so same results recorded by(Sammons *et al*.,2004).

Pseudomonas also showed a decline in numbers of viable organisms, survivors were present after 3 days on brushes, But no growth at 6 days as shown table 4 .

The persistence of viable *Staphylococcus* on drying toothbrush ,especially in the humid atmosphere of toothbrush holder ,is not suprising, since they can survive on hospital fabrics for several days(Neely & Maley, 2000) and both *Staphylococcus* and *Ps.aeruginosa* have been shown to survive in dried up films on non-nutrient surfaces ,cotton and blood protein coaqulum for several months (Smith *et al*.,1996). Most reports were showed bacterial colonization on toothbrushes by composition biofilm on them(Quirynen,2003) .

Several previous studies recommends healthy individual changing toothbrushes every three months, Sick children or adults should replace their toothbrushes as soon as possible to prevent reinfection or infection of another person (Glass&Lare,1986) .

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التشوب الجرثومي لفرش الأسنان ومقارنتها للأشخاص الأصحاء ومرضى الأسنان

الخلاصة

جمعت (24عينه)من فرش ألاسنان (12) منها لاشخاص اصحاء (12) أخرى لاشخاص مرضى وذلك بعد 5 أسابيع من أستخدام الفرش على الاقل .

كلتا عينات فرش المجموعتين أستوطنت بأعداد كبيرة من الجراثيم أذ تراوحت نتائج العد البكتيري في عينات فرش الاصحاء من $0,2 \times 10^2$ الى $3,5 \times 10^2$ الوحدات المكونه للمستعمره /مل وفي عينات فرش المرضى كانت من $2,8 \times 10^2$ الى 5×10^2 الوحدات المكونه للمستعمره /مل .

تميزت عينات فرش الاصحاء بنمو أنواع مختلفه من الجراثيم مثل المكورات العنقوديه الذهبيه و المكورات العنقوديه البيضاء والزوائف والخمائر وعصيات موجبه الغرام في حين عزل نوع واحد من الجراثيم في أغلب عينات فرش المرضى.

بينت نتائج التحليل الأحصائي أن هناك فرق معنوي($P<0.05$) بين فرش المجموعتين في المعدل الكلي للجراثيم المعزولة. سجلت جرثومه الزوائف نسبه (57٪) من مجموع الجراثيم المعزوله في عينات الفرش تلتها جرثومه العنقوديات (36٪) و سجلت عصيات موجبه الغرام أقل نسبه عزل(3٪).

أظهرت كلا المكورات العنقوديه الذهبيه و المكورات العنقوديه البيضاء القدره على البقاء على عينات فرش الاسنان المرضى المنتخبه بعد 6 أيام من التفريش أوالحفظ في حين سجلت الزوائف القدره على البقاء بعد 3 أيام من التفريش.بينت هذه الدراسه خطوره فرش الاسنان الملوثه كمستودع لعدد من الجراثيم الانتهازيه مثـــل العنقوديات والزوائف في اصابات الفم.